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IMPACT OF PHYTOHORMONES AUXIN AND CYTOKININ ON MAMMALIAN CELLS

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ABSTRACT

Phytohormones are chemical signals released in minimum concentrations by plant cells to perform various important physiological and biological functions. Auxins and cytokinins are the two major classes of phytohormones. Current study deals with the effect of these phytohormones on mammalian cells. Our results suggested that both IAA (auxin) and kinetin (cytokinin) enhances the cell density of HEK293 and NIH3T3 mammalian cell lines after treatment without compromising cell viability. We also showed that both hormones augmented cell cycle process in both cell lines by increasing the expression of cyclins and decreasing the expression of cyclin-dependent kinase inhibitor p21. Our results provide an insight into the impact of phytohormones on mammalian cells.

KEYWORDS: Auxins and Cytokinins & Phytohormones on Mammalian Cells

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Original Article

INTRODUCTION

Phytohormones are signaling molecules which regulate growth and development in plants (Neumann et al., 2009). These molecules are produced in very low amounts in cells and regulate its division and differentiation (Neumann et al., 2009). In general, phytohormones are divided into five major classes, namely auxins, cytokinins, ethylene, abscisic acid, gibberellins while the sixth class consists of all other phytohormones like brassinosteroids, salicylic acid, jasmonates, strigolactones and others (Kende and Zeevaart 1997). Each phytohormone have specific or an overlapping signaling pathway to perform their biological function (Srivastava 2002). Many plant hormones have shown medicinal activity. Sodium salicylate suppresses the proliferation and induce apoptosis in human cancer cells (Fingrut andFlescher 2002). Jasmonates are emerging as a new family of anti-cancer agents (Flescher 2005), they act directly and selectively on human cancer cell mitochondria (Rotem 2005).

Auxins and cytokinins are the two major classes of phytohormones their main function is cell division and differentiation. The dynamics of auxin and cytokinin play a key role in in-vitro plant propagation. Auxin promotes shoot formation while cytokinins promote root formation (Venkatachalam and Jayabalan 1997). Recently, Vildanova and Smirnova showed the effects of phytohormones on mammalian cells, either positively or negatively (Vildanova and Smirnova 2016). Soon after, Othman and their coworkers reported that cytokinins provide protection against oxidative stress in mammalian cells (Othman et al., 2016). The effects of plant hormones on mammalian cells have gained much attention still, the impact of major phytohormones on animal cell division is poorly understood.

In the current study, we showed the effects of phytohormones auxin and cytokinin on human fibroblast cell line

(NIH3T3) and human embryonic kidney cell line (HEK293). We analyzed the effects of both hormones on animal cell division and growth. Additionaly, the expression of cell cycle regulators after phytohormones treatment was also investigated.

MATERIALS AND METHODS

Chemical

Indole-3-acetic acid (IAA) and Kinetin were purchased from Sigma. Media for animal cell culture was purchased from Himedia while PBS was purchased from Sigma. Animal cell lines (HEK293 and NIH3T3) were originally purchased from ATCC and maintained in the laboratory.

IAA and Kinetin Treatment

HEK293 and NIH/3T3 cells were grown up to 75 % confluency in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) to provide embryonic growth promoting factors. Then, cells were trypsinized and seeded in equal volume. Kinetin and IAA (100 nM) were added individually into growth medium cells were incubated at 37^0C for next 48 hrs at 5% CO_2 in humidified incubator...

Trypan Blue Exclusion

Cell was trypsinized after 48 hrs and 0.1 ml of cells were stained with 0.1 ml of0.4% trypan blue (prepared in PBS, pH 7.2). Cells were mixed gently and loaded quickly on a hemocytometer to examine under the light microscope at low magnification. The total number of blue stained cells were counted. The percentage of viable cells were counted by following formula,

% viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100

Cell Cycle Analysis

Cell cycle analysis was performed by flow cytometry using propidium iodide staining. Cells were treated with IAA and Kinetin as described above. Followed by trypsinization, and washing with 1X PBS washing (twice)... Cells were fixed in 70 % ethanol for 30 min at 4^0C followed by double washing in PBS. The cells were resuspended in a 200µl 1X PBS solution containing 50µl RNase (100µg/ml) to ensure that only DNA was stained and propidium iodide (50µg/ml). Followed by incubation in the dark for 30 mins. Analysis was done by flow cytometer at the maximum emission of 605nm.

Analysis of Cell Cycle Regulators

A cells cycle regulator like cyclins and cyclin-dependent kinase inhibitors, which positively and negatively regulate cell cycle respectively, were analyzed by western blotting with the specific monoclonal antibody. Cells were treated with IAA and kinetin,. After 48 hrs cell lysate was prepared, resolved in 12 % SDS-PAGE gel, and transferred to PVDF membrane followed by blocking with 5%BSA. Thereafter, the membrane was washed with PBST buffer and incubated with the desired antibody (cyclin d1 and p21) at 2000 times dilution at 4 (overnight) hrs,. Blots were washed subsequently with TBST and incubated with secondary antibody at 10,000 times dilution for 2hrs at room temperature. Blots were visualized by the HRP detection method.

Statistical Analysis

All the experiments were performed in triplicates on three biological replicates. The data were analyzed by One

way-ANOVA (P <0.05) by using Graph Pad Prism 7.0 software. Data were shown as mean with standard deviation error bars.

RESULTS

IAA and Kinetin Enhances the Cell Density

HEK293 and NIHT3T3 cells were treated with 100 nM of IAA and Kinetin separately. We observed that confluency of both cell line was increased and 48 hrs of treatment in comparison to control cells (Figure 1).

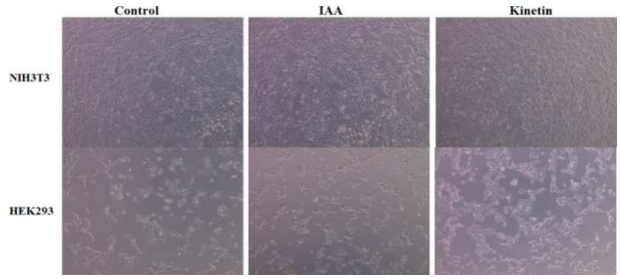

Figure 1: Representative Image of HEK293 and NIH3T3 After IAA and Kinetin Treatment

We further measured the total number of cells on a hemocytometer, our results showed that after the treatment of IAA and kinetin the total number of cells in both cell lines were significantly increased as compared to untreated controls. The total number of HEK293 and NIH3T3 cells after IAA treatment were 1.5 and 1.9 folds higher over control, respectively (Figure 2). Kinetin treatment also enhanced 2.0 and 2.9 fold change in total number of HEK293 and NIH3T3 cells, respectively in than control cells (Figure 2). Further Trypan Blue Exclusion assay, confirmed that over 95 % cells were viable in both treatments as well as control.

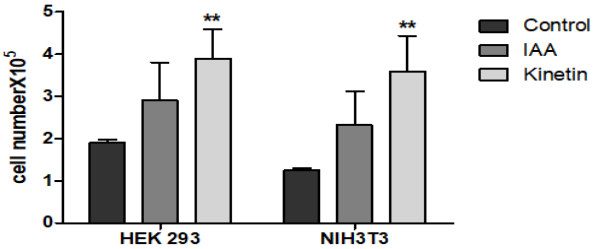

Figure 2: Graph Showing the Total Number of Cell Population After IAA and Kinetin Treatment

IAA and Kinetin Boost Cell Division of Mammalian Cells

Cell cycle analysis of both mammalian cell lines by flow cytometry revealed that, IAA and kinetin both augmented cell cycle progression. Approximate 58% cells population in both cell lines were in G1 phase of the cell cycle after 48 hrs without any treatment (Figure 3). IAA and kinetin decrease the proportion of this G1 population and increased S phase and G2M phase cell populations. The NIH/3T3 cells showed the maximum decline in G1 phase population after kinetin treatment. Kinetin reduced 22.54% and 31.06 % G1 cell population of HEK293 and NIH3T3 over control while IAA reduces 17.60 and 15.73 % of the G1 population in same cell lines, respectively (Figure 3). On the other hand, Both IAA and Kinetin enhanced the cumulative population of S and G2M phases in both cell lines. S and G2M phase cumulative population ofHEK293 cells were increased by 24.76 and 31.72 % over control after IAA and kinetin

treatments, respectively. In the NIH3T3 cells, the same cumulative population was increased by 22.09 and 43.06 % in comparison to control after IAA and kinetin treatments, respectively (Figure 3).

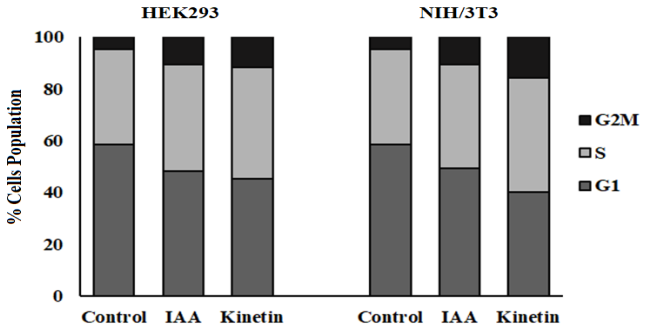

Figure 3: Graph Showing the Individual Proportion of Each Cell Cycle Stage after IAA and Kinetin Treatment

IAA and Kinetin Increases the Expression of Cyclins

Cyclins are the positive cell cycle regulators in the cells. Our result suggested that the expression of cyclin D1 was enhanced after IAA and kinetin treatment in both HEK293 and NIH3T3 cell lines. The expression of β-actin, which was used as an internal control, was constant after treatment. We also analyzed the expression of negative cell cycle regulator p21, (a cyclin-dependent kinase inhibitor). The treatment of both IAA and kinetin decreased the expression of p21 in both the cell lines(Figure 4).

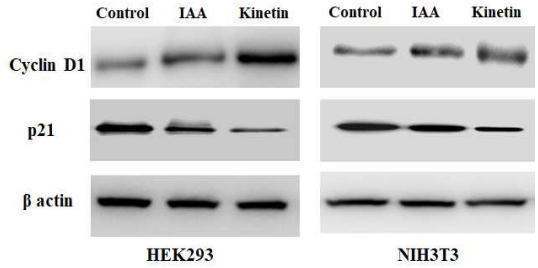

Figure 4: Western Blot Showing differential Expression of Cyclin D1 and p21 after IAA and Kinetin Treatment

DISSCUSIONS

Auxins and cytokinins are important phytohormones families. They performed many important biological and physiological functions in plants, like phototropism, gravitropism, shade avoidance response, vegetative growth, apical dominance, fruit development and others (Werner and Schmülling, 2009;Zhao, 2010). The main function of Auxins and cytokinins in plant cell is to regulate cell division and differentiation (Werner and Schmülling, 2009;Zhao, 2010). Biosynthesis, perception and signaling pathway of auxin and cytokinin are well reported. Recently, researchers showed that phytohormones are able to effect on the biological processes in mammalian cells (Vildanova and Smirnova, 2016; Othman et al., 2016). So, we analyzed the effect of auxin and cytokinin in mammalian cells. We selected IAA and kinetin as a representative member of auxin and cytokinin due to their biological origin (Amasino, 2005; Zhao, 2010). IAA and kinetin both promote cell division and differentiation in plant cells (Davies, 2010), our preliminary results also demonstrated that total number of mammalian cells are enhanced after IAA and kinetin treatment without compromising cell viability. This suggests that both IAA and kinetin have a positive effect on cell growth. The next obvious question is

the status of the cell cycle. So we analyzed cell cycle events by flow cytometry after IAA and kinetin treatment. Both IAA and kinetin augmented cell cycle progression by decreasing G1 population and increasing the S and G2M population. This suggested that more cell is in dividing phase after IAA and kinetin treatment. Although we observed the differential effect between mammalian cell line HEK293 and NIH3T3. NIH3T3 was found to be more responsive than HEK293, this might be due to their different biological origin. A cell cycle is the highly regulated molecular event. It is checked and regulated via several proteins through distinct mechanisms (Galderisi et al., 2003). Cyclins play a key role in cell cycle progression, presences or absence of cyclins protein decides the fate of stages in the cell cycle, Moreover, each stage is governed by at least one cyclin (Galderisi et al., 2003). Our results suggested that both IAA and kinetin enhanced the expression of cyclin D in both mammalian cell lines. Cyclin D is responsible for G1/S phase transition (Lodish et al., 2012), and we also observed that IAA and kinetin treatment promotes the higher transition from G1 to S phase. This suggests that both results complement each other. $p21^{cip1}$is a cyclin-dependent kinase inhibitor, which negatively regulates cell cycle by reducing cyclin D activity (Cheng et al., 1999). IAA and kinetin decrease the expression of p21, which might be the reason for high cyclin D activity.

Collectively, our results concluded that the IAA and Kinetin somehow decreased the expression of p21 in mammalian cells, which resulted in higher cyclin D activity in cells. Cyclin D is responsible for G1/s phase transition and augments cell cycle, thus resulted into higher cell density. Our study provides an insight into the effects of auxins and cytokinins on mammalian cells, but also open new queries like perception and detailed signaling of phytohormones in the mammalian system, which need to study in the future.

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